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Direct Tissue Profiling of Protein Complexes: Toward Native Mass Spectrometry Imaging

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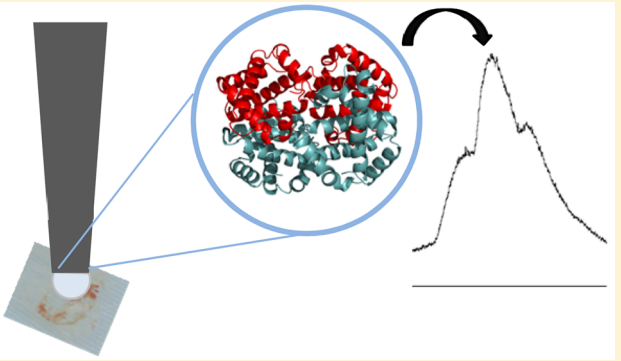
Supporting Information

ABSTRACT: Native mass spectrometry seeks to probe noncovalent protein interactions in terms of protein quaternary structure, protein−protein and protein−ligand complexes. The ultimate goal is to link the understanding of protein interactions to the protein environment by visualizing the spatial distribution of noncovalent protein interactions within tissue. Previously, we have shown that noncovalently bound protein complexes can be directly probed via liquid extraction surface analysis from dried blood spot samples, where hemoglobin is highly abundant. Here, we show that the intact hemoglobin complex can be sampled directly from thin tissue sections of mouse liver and correlated to a visible vascular feature, paving the way for native mass spectrometry imaging.



Native mass spectrometry is an emerging field in which structural information about intact protein complexes may be obtained from gas-phase data. A requirement of the approach is that the protein fold is maintained during and after transfer from solution to the gas phase. The preservation of noncovalent bonding in protein−ligand complexes in the gas phase was first demonstrated by Ganem et al.[1] Shortly after, Katta and Chait reported the observation of intact complexes of myoglobin and the prosthetic heme group following electrospray ionization.[2] Subsequent work by Breuker and McLafferty showed that electrospray ionization enables the preservation of native structures (inter- and intramolecular interactions).[3] Native mass spectrometry is now being widely applied to the interrogation of protein assemblies, particularly their stoichiometry, architecture, and stability.[4−9] The interest in the approach is fueled by the recognition that proteins do not typically exist in isolation. Sobott and co-workers[10] have estimated that half of all cellular proteins form complexes, either as oligomeric assemblies or as transient interacting partners of other proteins.

A parallel emerging branch of biological mass spectrometry is that of mass spectrometry imaging.[11] Mass spectrometry imaging involves analysis of molecules directly from tissue thus preserving spatial information and allowing, for example, the determination of molecular signatures characteristic of healthy and diseased states. The majority of mass spectrometry imaging experiments employ matrix-assisted laser desorption/ionization (MALDI), which typically produces singly charged ions, coupled with time-of-flight (TOF) mass analyzers. A limitation of MALDI mass spectrometry imaging of intact proteins is protein identification, both as a result of poor fragmentation of singly charged precursor ions and low resolution of the TOF analyzer, although the latter can be addressed for low molecular weight proteins by use of Fourier transform ion cyclotron resonance mass spectrometry.[12] Furthermore, MALDI analysis of noncovalent complexes is nontrivial and, unlike electrospray, cannot be reliably linked to solution-phase structure.[13] More recently, desorption electrospray ionization (DESI) has been applied for mass spectrometry imaging; however, to date, DESI analysis of solid substrates has not proved useful for proteins of molecular weight >25 kDa.[14−16] A variant of DESI, known as liquid sample DESI, in which proteins up to 150 kDa have been desorbed from solution, has been demonstrated.[17]

An alternative approach is liquid extraction surface analysis (LESA) mass spectrometry.[18] LESA is a direct surface sampling technique in which a biological substrate is sampled via a liquid microjunction which is subsequently reaspirated and introduced to the mass spectrometer via electrospray ionization. LESA mass spectrometry has been applied widely for the analysis of proteins in non-native conditions,[19−24] including identification of unknown proteins from tissue sections and unknown hemoglobin variants from dried blood spots by MS/MS.[20,22−24] By adjusting the composition of the sampling/electrospray solution, we have shown that native LESA of protein complexes can be demonstrated for proteins spotted onto glass and polymeric substrates and for dried blood spots.[25]

In this Letter, we describe the marriage of native mass spectrometry with spatially defined LESA sampling of thin tissue sections, thus paving the way for native mass spectrometry imaging. We show native LESA mass spectrom-

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etry of thin tissue sections of mouse liver and correlate observation of the tetrameric hemoglobin complex with a vascular feature within the tissue. Native hemoglobin exists as a noncovalently bound tetramer of two α-globin chains and two β-globin chains. Each globin subunit is noncovalently bound to a heme group. The tetramer assembles via association of two $\alpha^{H}\beta^{H}$ dimers (where H is the heme group).[26]

Figure 1A shows the results obtained following LESA sampling of the bulk liver tissue with the native solvent system.

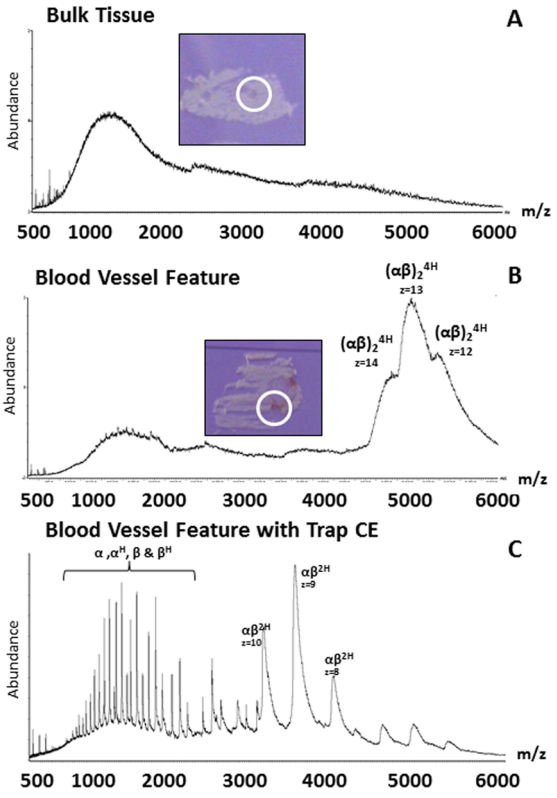

Figure 1. Native LESA mass spectrometry of thin tissue sections of mouse liver. Sampling was performed by contact LESA.[28] (A) Native LESA mass spectrum obtained after sampling of the bulk tissue. Inset: Photograph of tissue section postsampling. Sampling location is indicated. No resolved peaks corresponding to proteins are observed. (B) Native LESA mass spectrum obtained after sampling of the vascular feature. Inset: Photograph of tissue section postsampling. Sampling location is indicated. Peaks corresponding to the intact hemoglobin tetramer, $(\alpha\beta)_2^{4H}$, are observed. (C) Native LESA mass spectrum obtained after sampling of the vascular feature coupled with collisional activation (20 V) in the trap traveling wave ion guide. Peaks corresponding to α- and β-globin monomers, heme-bound α^{H} and β^{H} monomers, and heme-bound $(\alpha\beta)^{2H}$ heterodimers are observed.

The site of LESA sampling is indicated on the photograph inset. No peaks corresponding to proteins are observed in the mass spectrum. Previous experiments on mouse liver tissue have shown that a variety of protein species can be extracted and detected by LESA mass spectrometry with denaturing solvent systems.[22,23] The differences between those mass spectra and that obtained here from bulk tissue with native solvent (Figure 1A) may be due to differences in LESA extraction efficiency of the various solvent systems.

A set of serial sections of the mouse liver reveal a vascular feature; see insets of Figures 1B and 2. LESA sampling of the liver tissue at a location where the vascular feature is evident (see Figure 1B inset photograph) revealed peaks at $m/z \sim$ 4500, 4900, and 5300 in the mass spectrum corresponding to the hemoglobin tetramer $(\alpha\beta)_2^{4H}$ in the 14+, 13+, and 12+ charge states, respectively. (For comparison, a direct infusion electrospray mass spectrum of hemoglobin standard in the native solvent system is shown in Supporting Figure 1A). In each case, the peak width is broad (~250 Th). That observation is likely due to solvent and buffer molecules which remain bound to the complex, in addition to salt adducts originating from the tissue, and is consistent with previous examples of native hemoglobin tetramers from biological samples.[25,27]

To confirm the identity of these species, the ions were collisionally activated in the trap traveling wave ion guide. The resulting mass spectrum is shown in Figure 1C. A number of peaks were observed between m/z 1000 and 2700. Peak assignments are summarized in Table 1. Peaks at m/z 1153, 1249, 1363, 1499, 1665, 1873, 2141, and 2498 correspond to α-globin in the 13+ to 6+ charge states. Similarly, peaks at m/z 1202, 1302, 1420, 1562, 1736, 1953, 2232, and 2603 correspond to β-globin in charge states +13 to +6. Peaks at m/z 1560, 1734, 1950, 2228, and 2599 correspond to 10+ through 6+ charge states of α-globin bound to heme (α^{H}), and those at m/z 1624, 1804, 2030, and 2319 to the 10+ through 7+ charge states of β^{H}. Peaks at $m/z \sim$ 3200, 3500, and 4000 correspond to the 10+, 9+, and 8+ charge states of the $(\alpha\beta)^{2H}$ dimer. As mentioned above, formation of the hemoglobin complex proceeds via association of two $(\alpha\beta)^{2H}$ dimers. Observation of the heterodimer, but not the homodimers, in our experiments is in agreement with that finding. For comparison, a direct infusion native ESI mass spectrum of hemoglobin protein standard obtained following collisional activation in the trap traveling wave ion guide is shown in Supporting Figure 1B.

The results obtained following collisional activation confirm the assignment of the hemoglobin tetramers. Moreover, observation of a peak corresponding to the 13+ charge state of $(\alpha\beta)_2^{4H}$ in the intact mass spectrum (Figure 1B) is particularly important. The m/z of a tetramer with an even charge state, $2n+$, is identical to that of a dimer with charge state $n+$; however, that is not true for tetramers with odd charge states, and detection of the 13+ species confirms the presence of the tetramer.

Figure 2 shows the mass spectra obtained following native LESA sampling of two locations within the vascular feature on four serial sections. Mass spectral intensities are normalized to that shown in Figure 2C, location 2. Photographs show the tissue sections pre- sampling together with sampling locations. Sampling locations indicated correspond to sampling area. In each case, peaks corresponding to hemoglobin tetramer ions are observed. The peaks are observed in greater abundance at location 2 which is in agreement with that anticipated on the basis of visual inspection of the tissue sections. Two challenges for native LESA sampling that are apparent from our results are (1) determination of the extraction efficiency of native LESA sampling and (2) the need for improved spatial resolution. The sampling areas indicated correspond to the inner diameter of the sampling pipet tip, i.e., diameter of 400 μm and sampling area of 0.126 mm^2 assuming solvent spread is limited by the pipet walls. (In separate experiments, optical imaging of brain tissue that had previously been sampled with contact-LESA using an organic solvent system suggests that there may be some solvent spread, but it is unclear whether this is during or

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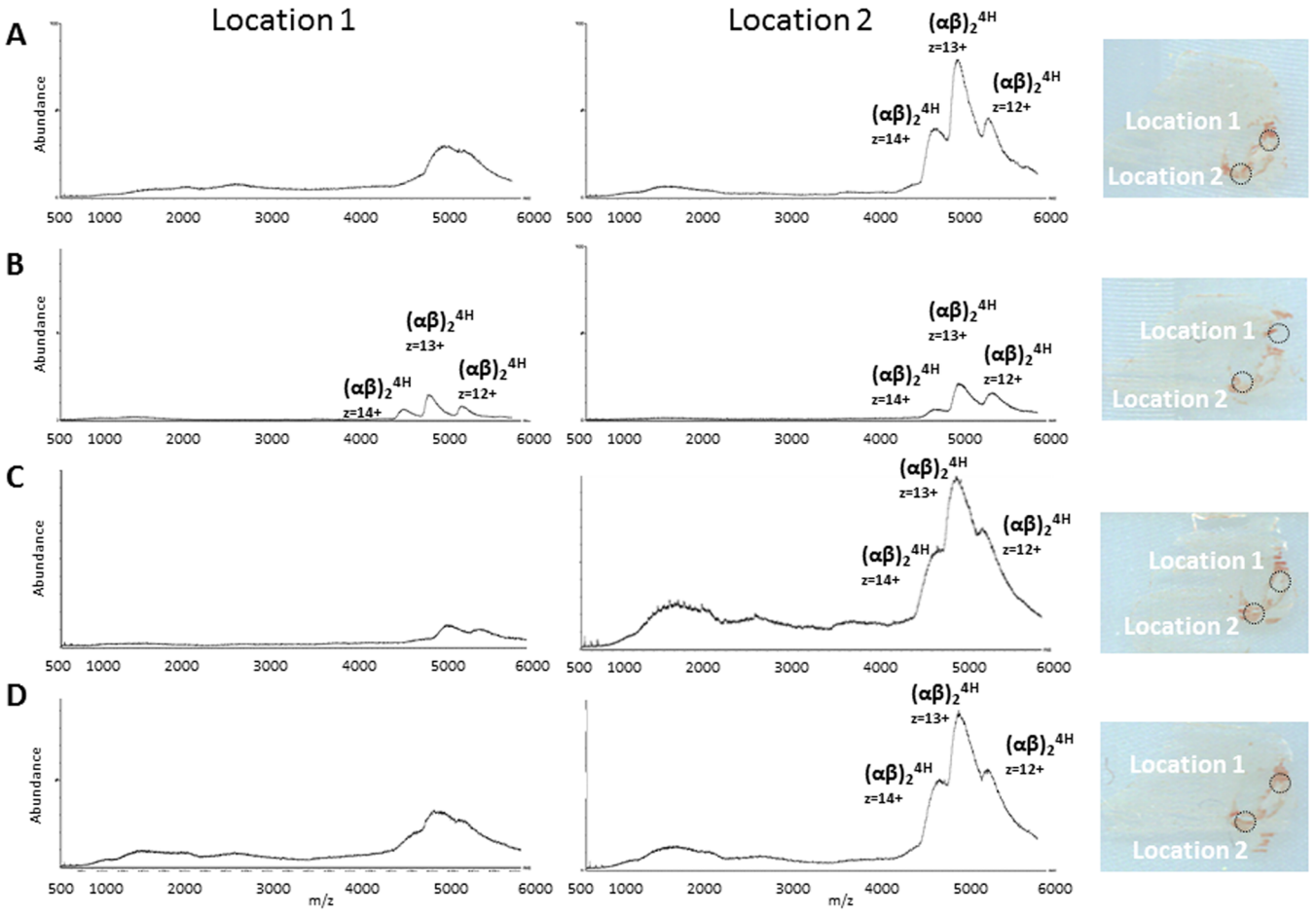

Figure 2. (A–D) Native LESA mass spectrometry of specific locations in four serial sections of mouse liver. Visual inspection of the tissue sections reveals the presence of a vascular feature. Native LESA sampling at discrete locations within the vascular feature results in the detection of tetrameric hemoglobin complexes $(\alpha\beta)_2^{4H}$ in multiple charge states. Mass spectra are normalized to the base peak of the mass spectrum shown in (C) location 2. Inset: Photographs of the tissue sections presampling. LESA sampling area is indicated and corresponds to the inner diameter of the sampling pipet tip (diameter = 400 μm).

Table 1. Peak Assignments Following Collisional Activation of Hb Tetramers in the Trap Traveling Wave Ion Guide

measured m/z	calculated m/z	assignment
1153, 1249, 1363, 1499, 1665, 1873, 2141, 2497	1153, 1249,1363, 1499, 1665, 1873, 2141, 2498	α^{13+}, α^{12+}, α^{11+}, α^{10+}, α^{9+}, α^{8+}, α^{7+}, α^{6+}
1202, 1302, 1420, 1562, 1736, 1953, 2232, 2603	1202, 1302, 1420, 1562, 1735, 1953, 2232, 2603	β^{13+}, β^{12+},β^{11+}, β^{10+}, β^{9+}, β^{8+}, β^{7+}, β^{6+}
1560, 1734, 1950, 2228, 2599	1559, 1733, 1949, 2228, 2599	α^{H10+}, α^{H9+}, α^{H8+}, α^{H7+}, α^{H6+}
1624, 1804, 2030, 2319	1624, 1804, 2030, 2320	β^{H10+}, β^{H9+}, β^{H8+}, β^{H7+}
~3200, ~3500, ~4000	3184, 3538, 3980	$(\alpha\beta)^{2H\ 10+}$, $(\alpha\beta)^{2H\ 9+}$, $(\alpha\beta)^{2H\ 8+}$

postsampling (data not shown).) Higher spatial resolution would require reduced sampling area; however, the current spatial resolution provides high sensitivity and allows coarse structural features to be interrogated.

In conclusion, we have shown the location specific profiling of a noncovalent protein complex directly from tissue. The implications of this are manifold: Native mass spectrometry imaging could inform on protein architecture, signaling, and drug metabolism *in situ*. Questions that might be addressed could include, for example, in what regions of tissue is a drug binding to which protein? Nevertheless, a number of challenges need to be addressed for the approach to be broadly applicable. This work has focused on the abundant hemoglobin tetramer. Native mass spectrometry imaging of less abundant or less soluble protein complexes will require improvements in sensitivity and LESA extraction efficiency. Further work in that area is ongoing.

EXPERIMENTAL SECTION

Samples. Liver from wild-type mice (extraneous tissue from culled animals) were the gift of Prof. Steve Watson (University of Birmingham). Organs were frozen on dry ice and then stored at −80 °C until sectioned. Sections of murine liver tissue were obtained at a thickness of 10 μm using a CM1810 Cryostat (Leica Microsystmes, Wetzlar, Germany) and thaw mounted onto glass slides. Hemoglobin protein standard was purchased from Sigma-Aldrich (Gillingham, UK) and used without further purification.

Surface Sampling. "Contact" LESA as described in ref 28 was carried out by use of the Triversa Nanomate (Advion Biosciences, Ithaca, USA) coupled with the Synapt G2S mass

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spectrometer. The sample was mounted onto the LESA universal adaptor plate, and an image was acquired using an Epson Perfection V300 photo scanner. The exact location to be sampled was selected using the LESA Points software (Advion). The extraction/ionization solvent was 95:5 ammonium acetate (10 mM, pH 6.8)/methanol (HPLC grade, JT Baker, Deventer, The Netherlands). In the extraction process, 10 μL of solvent was aspirated from the solvent well; then, the robotic arm was relocated to a position above the tissue section. The tip descended to a depth such that it was in contact with the tissue. Once in contact, 4 μL of solvent was dispensed and held in contact for 10 s. 4.5 μL of solvent was reaspirated and infused into the mass spectrometer at a gas pressure of 0.3 PSI and 1.5 kV.

Direct Infusion of Hb Standard. A 1 mg mL^{-1} solution of hemoglobin was prepared in ammonium acetate (20 mM, pH 6.8). Five microliter aliquots were introduced into the mass spectrometer via the Triversa Nanomate in positive mode at a gas pressure of 0.30 PSI and an ionization voltage of 1.6 kV.

Mass Spectrometry. All data were acquired in resolution mode without traveling wave ion mobility. The scan time was 5 s, and m/z range was 500−6000. The capillary temperature was set to 30 °C, and the cone voltage was set at 45 V. Data were acquired for 2−5 min in full scan mode. Collisional activation experiments were performed in the trap traveling wave ion guide at normalized collision energy of 20 V. Data were analyzed by use of the Mass Lynx software (version 4.1, Waters). Mass spectra were processed using the smooth function in Mass Lynx (mean of 50 channels × 2).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b03993.

Direct infusion native ESI mass spectra of hemoglobin protein standard, with and without collisional activation in the trap traveling wave ion guide. (PDF)

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Notes

The authors declare no competing financial interest.

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